9706061 Transgenic Fish: Development Of Models To Assess Risk By W. M. Muir, Ph.D., Department Of Animal Sciences and R. D. Howard, Ph.D., Department Of Biological Sciences, Purdue University

Specific aims - The long-term objective of this research is to determine if the fate of a transgenic organism introduced into a natural population could be predicted by a computer model based on fitness parameters of the base population and transgenic population as measured in a secure laboratory setting. This objective involves three stages: estimation, prediction, and testing. the first two and part of the third stage were the goals of this proposal: 1) Based on theoretical considerations, determine what parameters need to be estimated to predict risk and developed alternative methods to estimate those parameters, 2) developed a mathematical model which incorporates those parameters to predict risk, and 3) created different transgenic founder lines to test predictions of the model.

Results –

Parameter Estimation. Our research clearly showed that, to assess the ecological risk of a particular transgenic line, data on six net fitness factors are required: viability, fecundity, fertility, mating success, age at sexual maturity, and longevity. All biological factors that can influence the fitness of a transgenic animal fall into one of these categories. Our results stressed the importance of measuring all six factors because a disadvantage in one category can be offset by an advantage in another category. For example, reduced viability of transgenics could be offset by any one of the following size- or growth-related advantages of transgenics: 1) a reduced generation interval which would increase reproductive rate; 2) increased mating success of males; 3) increased egg production by females, and 4) reduced cannibalism on offspring.

Mathematical Model: To determine the extent to which any one of these fitness factors could offset a viability disadvantage, quantitative values of the factor for the specific transgenic line under investigation need to be put into our mathematical model. Our model brings together all six factors, using population genetics theory, to predict changes in transgene frequency and population size. Fortunately, it is not necessary to know why a transgenic line differs from its wild type counterparts in some fitness factor, only that a difference exists. For example, consider some viability difference: all that the model requires is the relative number of transgenics and wild types that reach sexual maturity; no information is needed on whether observed trends result from differences in disease resistance, foraging ability, escape from predation, improper gill size, or swimming ability, etc. Our model only requires the bottom line, the degree to which the two types differ. The underlying cause of the difference may be of academic interest, but may require extensive study and funding to determine. Thus, our model can be realistically implemented as a risk assessment tool by regulatory agencies and transgenic fish developers at minimal cost. .

Testing: Phase 1, Development of Alternative Founder Lines. We used Japanese medaka, *Oryzias latipes*, as a model study organism to explore the ecological consequences of transgenic release into natural populations. Medaka were convenient study organisms on which to obtain data on fitness components and propagate multiple generations within a relatively short period of time. Individuals readily bred in the lab, were easily cultured, and attained sexual maturity in about two months. We produced a stock of transgenic medaka by using cytoplasmic microinjection to insert a gene construct consisting of the human growth hormone gene (hGH) with a salmon promoter (sGH) into just fertilized eggs. Results of several experiments showed that: transgenic young had a viability disadvantage (survival was 69% of that of wild type); the transgenic line had a distinct early developmental advantage (peaking at 4 weeks of age with a 39% size advantage) which resulted in a 21% increase in escape from predation; and transgenic females produced 27% more eggs/spawn than wild type fish.

We also used cytoplasmic microinjection to inject a gene construct consisting of an all fish growth-hormone construct provided by Dr. R. H. Devlin (West Vancouver Lab, Dept. of Fisheries and Oceans, Gov't of Canada) consisting of the metallothionine promoter driving the salmon growth hormone gene. From 3000 injected eggs, we produced 17 founders, 3 of which bred true. These 3 founders were backcrossed to the base population and, in the F2, F3, and F4 generations, the 3 resultant lines were tested for viability, developmental time, fertility, and fecundity. Two of the three lines differed greatly in fitness characteristics relative to the base population from which they were derived. We were therefore successful in accomplishing our goal of obtaining founder lines with different trajectories.

Predictions: For the SGH-hGH line Our model predicted that, for a wide range of parameter values including those we documented experimentally, transgenes should spread in invaded populations despite high viability costs whenever the transgenes also have positive effects on fecundity or developmental rate. The other two lines (those derived from the all-salmon gene construct) are predicted to be eliminated if released into a wild population

We also examined the risk to a natural population after a release of a few transgenic individuals when the transgene simultaneously increased transgenic male mating success and lowered transgenic offspring viability. Mating experiments using wild type medaka were performed to assess the degree to which large males obtained a mating advantage over small males. We found that large males obtained a four-fold mating advantage. Large male mating advantages have also been reported in several salmonid species. Our deterministic equations predict that a transgene introduced into a natural population by a small number of transgenic fish will spread rapidly as a result of enhanced mating advantage, but the reduced viability of transgenic offspring will cause eventual local extinction of both the wild and transgenic populations. Furthermore, loss of a wild type population could also have cascading, negative effects on the rest of the ecological community. This type of risk has not been previously described.

Plans for the coming year – In the coming year we will finalize our results for estimating mating success using dynamic estimation methods in all three lines. The final stage of our research involves completion of the third stage: testing alternative predictions of models using alternative founder lines and parameters estimated in the first stage to test predictions of the second stage and is the objective of a recently submitted grant.

Publications Resulting From Grants Supported by this Program to Date

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3. Muir, W. M., R. D. Howard, R. S. Martens, S. Schulte, and C. A. Bidwell. 1996. Use Of Multi-generational studies to assess genetic stability, fitness, and competitive ability of transgenic Japanese medaka: III. Results and predictions *in* M. Levin, C. Grim, and J. Angle eds. Proc. of 8th International Conference on Risk Assessment Methodologies.

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5. Muir, W. M. and R. D. Howard. 1999. Possible ecological risks of transgenic organism release when transgenes affect mating success: sexual selection and the Trojan gene hypothesis. Proc. Natl. Acad. Sci. 96: 13853-13856.

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